FORM PTO-1390 (REV 10-94) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 9555.117USWO

US APPLICATION NO (If known, see 37 C F R 1 5): 09/831075

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA99/01065	November 8, 1999	November 6, 1998

TITLE OF INVENTION

IMPROVED BACTERICIDAL AND NON-BACTERICIDAL SOLUTIONS FOR REMOVING BIOFILMS

APPLICANT(S) FOR DO/EO/US

Jean BARBEAU; Denis GRAVEL; Abdelkrim HABI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [X] An unsigned oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [ ] Other items or information:

JC08 Rec'd PCT/PTO 03 MAY 2001

U.S. APPLICATION NO (If known, see 37 C F R 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/831075	PCT/CA99/01065	9555.117USWO

17. [X] The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):

Search Report has been prepared by the EPO or JPO **$860.00**

International preliminary examination fee paid to U.S. Patent and Trademark Office (37 CFR 1.492(a)(1)) **$690.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO **$1000.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				**$860**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				**$**	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	
Total claims	40	-20 =	20	X **$18.00**	**$360**
Independent claims	5	-3 =	2	X **$80.00**	**$160**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ **$270.00**	**$**
TOTAL OF ABOVE CALCULATIONS =					**$1380**
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					**$690**
SUBTOTAL =					**$690**
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f).				+	**$**
TOTAL NATIONAL FEE =					**$690**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property				+	**$**
TOTAL FEES ENCLOSED =					**$690**
				Amount to be: refunded	**$**
				charged	**$**

a. [X] Check(s) in the amount of $690 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
Gregory A. Sebald
MERCHANT & GOULD
P.O. Box 2903
Minneapolis, MN 55402-0903

SIGNATURE

Gregory A. Sebald
NAME

33,280
REGISTRATION NUMBER

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN	Docket No. 9555.87-US-WO

Serial No.	Filing Date	Patent No.	Issue Date
09/831,075	May 3, 2001		

Applicant/
Patentee: **Jean BARBEAU, Denis GRAVEL and Abdelkrim HABI**

Invention: **Improved Bactericidal and Non-Bactericidal Solutions for Removing Biofilms**

I hereby declare that I am:

- ☐ the owner of the small business concern identified below:
- ☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: **Theratechnologies Inc.**

ADDRESS OF CONCERN: **2310 boulevard Alfred-Nobel, Saint-Laurent, Québec, CANADA H4S 2A4**

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:

- ☐ the specification filed herewith with title as listed above.
- ☒ the application identified above.
- ☐ the patent identified above.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☐ no such person, concern or organization exists.
- ☒ each such person, concern or organization is listed below.

FULL NAME **Université de Montréal**
ADDRESS **2900 boulevard Édouard-Montpetit, Montréal, Québec, CANADA H3C 3J7**

☐ Individual ☐ Small Business Concern ☒ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: JACQUES M. SAINT-DENIS
TITLE OF PERSON SIGNING OTHER THAN OWNER: VICE PRESIDENT
ADDRESS OF PERSON SIGNING: CORPORATE AFFAIRS AND SECRETARY
THERATECHNOLOGIES INC
2310 ALFRED-NOBEL BLVD.
SAINT-LAURENT- QUEBEC- H4S 2A4

SIGNATURE: J.M. Saint-Denis VICE PRESIDENT DATE: JUNE 22, 2001

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (d)) - NONPROFIT ORGANIZATION	Docket No. 9555.87-US-WO

Serial No.	Filing Date	Patent No.	Issue Date
09/831,075	May 3, 2001		

Applicant/ Patentee: **Jean BARBEAU, Denis GRAVEL and Abdelkrim HABI**

Invention: **Improved Bactericidal and Non-Bactericidal Solutions for Removing Biofilms**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: **Université de Montréal**

ADDRESS OF ORGANIZATION: **2900 boulevard Édouard-Montpetit**
Montréal, Québec
CANADA H3C 3J7

TYPE OF NONPROFIT ORGANIZATION:

- ☒ University or other Institute of Higher Education
- ☐ Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3))
- ☐ Nonprofit Scientific or Educational under Statute of State of The United States of America
  Name of State: Citation of Statute:
- ☐ Would Qualify as Tax Exempt under Internal Revenue Service Code (26 U.S.C. 501(a) and 501(c)(3)) if Located in The United States of America
- ☐ Would Qualify as Nonprofit Scientific or Educational under Statute of State of The United States of America if Located in The United States of America
  Name of State: Citation of Statute:

I hereby declare that the above-identified nonprofit organization qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees to the United States Patent and Trademark Office regarding the invention described in:

- ☐ the specification to be filed herewith.
- ☒ the application identified above.
- ☐ the patent identified above.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the above-identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☐ no such person, concern or organization exists.
- ☒ each such person, concern or organization is listed below.

FULL NAME **Theratechnologies Inc.**
ADDRESS **2310 boulevard Alfred-Nobel, Saint-Laurent, Québec, CANADA H4S 2A4**

☐ Individual ☒ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

FULL NAME
ADDRESS

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

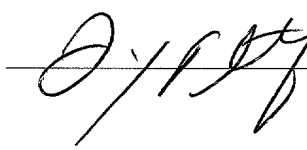
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Jean Yvon Timothy

TITLE IN ORGANIZATION: Directeur, Bureau de liaison entreprises-Université et des subventions

ADDRESS OF PERSON SIGNING:
Université de Montréal
Direction générale de la recherche
C.P. 6128, Succursale Centre-ville
Montréal, Québec
H3C 3J7

SIGNATURE:  DATE: June 19, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	BARBEAU, et al.	Docket No.:	9555.117USWO
Serial No.:	Unknown	Filed:	May 3, 2001
Int'l Appln No.:	PCT/CA99/01065	Int'l Filing Date:	November 8, 1999
Title:	IMPROVED BACTERICIDAL AND NON-BACTERICIDAL SOLUTIONS FOR REMOVING BIOFILMS		

CERTIFICATE UNDER 37 CFR 1.10:
"Express Mail" mailing label number: EL658339385US
Date of Deposit: May 3, 2001

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

By: Yolanda Gray
Name: Yolanda Gray

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment:

IN THE ABSTRACT

Insert the attached Abstract page into the application as the last page thereof.

IN THE SPECIFICATION

A courtesy copy of the originally-filed PCT specification is enclosed herewith, but the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

Please cancel all claims currently entered in the application and add the following new claims:

41. A method for removing a biofilm from a surface, which comprises the step of contacting said surface with a composition comprising an effective dislodging amount of a detergent and an effective dislodging amount of an acid or a salt of an acid, said salt being capable of displacing divalent cations present in the structure of said biofilm, with the proviso that said composition is not a mixture achieving an aqueous final concentration of SDS 1 % - 2 % and EDTA 1%, or SDS 1% - 2% and mandelic and lactic acids, each at an individual concentration of 1% or in a combined concentration of 2%, for a time sufficient to disloge said biofilm, all percentages representing weight per volume concentrations.

42. A method as defined in claim 41, further comprising a bactericidal amount of a bactericide.

43. A method as defined in claim 41, wherein said detergent is SDS, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any detergent having a biofilm dislodging potency substantially equivalent thereto.

44. A method as defined in claim 43, wherein said equivalent detergent is CPC or CPB at a concentration of at least about 0.5%.

45. A method as defined in claim 42, wherein said detergent is SDS, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any

detergent having a biofilm dislodging potency substantially equivalent thereto.

46. A method as defined in claim 45, wherein said equivalent detergent is CPC or CPB at a concentration of at least about 0.5%.

47. A method as defined in claim 41, wherein said acid is mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

48. A method as defined in claim 42, wherein said acid is mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

49. A method as defined in claim 41, wherein said salt or acid is an EDTA salt or acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any salt or acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

50. A method as defined in claim 42, wherein said salt or acid is an EDTA salt or acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any salt or acid having a biofilm dislodging potency substantially equivalent thereto at a

suitable working pH value.

51. A method as defined in claim 41, wherein said salt or acid is sodium mandelate or mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration range of at least about 0.1 % at a working pH value or any salt having a biofilm dislodging potency substantially equivalent thereto.

52. A method as defined in claim 42, wherein said salt or acid is sodium mandelate or mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration range of at least about 0.1 % at a working pH value or any salt having a biofilm dislodging potency substantially equivalent thereto.

53. A method as defined in claim 41, wherein said acid is one or more of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, mandelic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine.

54. A method as defined in claim 42, wherein said acid is one or more of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, mandelic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic,

glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine.

55. A method as defined in claim 42, wherein said bactericide is hydrogen peroxide or any bactericide having a bactericidal potency and host spectrum substantially equivalent thereto.

56. A method as defined in claim 55, wherein said equivalent bactericide is mandelic acid, phenol, sodium hypochlorite, CPC or CPB.

57. A method as defined in claim 56, wherein mandelic acid or salt, phenol, sodium hypochlorite, CPC or CPB achieves, once reconstituted in an aqueous solution, a concentration of at least 0.1%, 0.1%, 0.5%, 0.1% and 0.1 %, respectively.

58. A method as defined in claim 41, which further comprises a biofilm dislodging enhancer agent.

59. A method as defined in claim 42, which further comprises a biofilm dislodging enhancer agent.

60. A method as defined in claim 58, wherein said enhancer agent is a calcium chelator.

61. A method as defined in claim 59, wherein said enhancer agent is a calcium chelator.

62. A method as defined in claim 60, wherein said calcium chelator is EDTA in an acid or salt form which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any calcium chelator having a chelating potency substantially equivalent thereto.

63. A method as defined in claim 61, wherein said calcium chelator is EDTA in an acid or salt form which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any calcium chelator having a chelating potency substantially equivalent thereto.

64. A method as defined claim 58 wherein said enhancer agent is a chaotropic agent.

65. A method as defined claim 59 wherein said enhancer agent is a chaotropic agent.

66. A method as defined in claim 64, wherein said chaotropic agent is SDS which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any chaotropic agent having a chaotropic potency substantially equivalent thereto.

67. A method as defined in claim 65, wherein said chaotropic agent is SDS which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or

any chaotropic agent having a chaotropic potency substantially equivalent thereto.

68. A method for removing a biofilm from a surface comprising the step of contacting said surface with a composition, which comprises an effective dislodging amount of a detergent and an effective dislodging amount of an acid or a salt of an acid; said detergent being selected from sodium dodecyl sulfate, sodium n-decyl diphenylether disulfonate, sodium cocoyl sarcosinate, polyoxyethylene sorbitan monolaureate, cetylpyridinium bromide and cetylpiridinium chloride; said acid being selected from the group consisting of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic, ethylenediamine tetraacetic, N-(hydroxyethyl) ethylenediamine triacetic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine, with the proviso that said composition is neither a mixture achieving a final concentration of SDS 1 % - 2 % and EDTA 1%, of SDS 1% - 2% and mandelic and lactic acids, each at an individual concentration of 1% or in a combined concentration of 2%, of SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%, nor a mixture of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA, all percentages representing final weight per volume concentrations, for a time sufficient to dislodge said biofilm.

69. A method as defined in claim 68, further comprising a bactericide selected from mandelic acid, phenol, sodium hypochlorite, hydrogen peroxide, CPC and CPB.

70. A method for removing a biofilm from a surface comprising the step of contacting said surface with a composition, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1% but less than 1% SDS, about 0.1% - 1% acid or a salt of an acid and at least about 0.25% but less than 1% EDTA, said acid being selected one or more of 2-ketoglutaric, mandelic, iminodiacetic, mucic, glycolic, fumaric, L-aspartic, phosphoric, pyruvic, chloroacetic acids and DL-alanine, for a time sufficient to dislodge said biofilm.

71. A method as defined in claim 70, further comprising a bactericidal amount of a bactericide.

72. A method for removing a biofilm from a surface comprising the step of contacting said surface with a composition, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1% SDS, at least about 0.1% acid or a salt of an acid, and at least about 0.25% EDTA, said acid being of 2-ketoglutaric, iminodiacetic, mucic, glycolic, fumaric, aspartic, phosphoric, pyruvic, chloroacetic acids and alanine, for a time sufficient to dislodge said biofilm.

73. A method as defined in claim 72, further comprising a bactericidal amount of a bactericide.

74. A method as defined in claim 71, wherein said bactericide is hydrogen peroxide at a final concentration of about 5%, or phenol at concentration of at least about 0.1%, or sodium hypochlorite at concentration of at least about 0.5%, or CPC or CPB at concentration of at least

about 0.5%.

75. A method as defined in claim 73, wherein said bactericide is hydrogen peroxide at a final concentration of about 5%, or phenol at concentration of at least about 0.1%, or sodium hypochlorite at concentration of at least about 0.5%, or CPC or CPB at concentration of at least about 0.5%.

76. A method comprising the step of contacting said surface with a composition, which once reconstituted in an aqueous solution, achieves a final concentration of at least 0.5% CPC or CPB, 1% EDTA, 1% an acid or a salt of an acid selected from mandelic, glycolic, fumaric, citric and phosphoric acids or a mixture thereof, and a buffering agent to achieve a pH of about 7.5 or higher, for a time sufficient to dislodge said biofilm.

77. A method as defined in claim 41 wherein said composition achieves a final concentration of SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%.

78. A method as defined in claim 41 wherein said composition achieves a final concentration of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA.

79. A method as defined in claim 41, wherein said time is at least about one hour.

80. A method as defined in claim 41, wherein said time is comprised between about 1 and about 18 hours.

REMARKS

A new abstract page is supplied to conform to that appearing on the publication page of the WIPO application, but the new Abstract is typed on a separate page as required by U.S. practice.

Applicant respectfully requests that the preliminary amendment described herein be entered into the record prior to calculation of the filing fee and prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicant's primary attorney-of record, Gregory A. Sebald (Reg. No. 33,280), at (612) 336-4728.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: May 3, 2001

By _____
Gregory A. Sebald
Reg. No. 33,280

GAS/sef

ABSTRACT

This invention relates to compositions for removing biofilms from contaminated surfaces. The compositions minimally comprise a detergent and a salt or a salt-forming acid. These components act together for rapidly and efficiently dismantle biofilms. The compositions may also comprise a bactericide, for destroying bacteria. One of the preferred compositions achieves, when reconstituted in a determined volume of water, concentrations of 0.5% cetylpyridinium moieties, 1% EDTA and 1% of an acid or a salt of an acid selected from mandelic, glycolic, fumaric, citric and phosphoric acids, or a mixture thereof, at a pH higher than about 7.5. This composition removes and destroys biofilms.

TITLE OF THE INVENTION

IMPROVED BACTERICIDAL AND NON-BACTERICIDAL SOLUTIONS FOR REMOVING BIOFILMS

FIELD OF THE INVENTION

This invention relates to solutions capable of efficiently cleaning surfaces susceptible to biofilm coating thereon. It further relates to a cleaning/disinfecting solution, comprising the cleaning components and a bactericidal effective amount of a disinfectant.

BACKGROUND OF THE INVENTION

Bacteria in natural aquatic environments have the marked tendency to interact with surfaces. The formation of surface biofilms can be regarded as a universal bacterial strategy for survival and for optimum positioning with regard to available nutrients. Bacteria growing in natural environments produce extensive exopolysaccharide (EPS) polymers that mediate both their attachment to surfaces and the formation of microcolonies and, eventually, the generation of biofilms. Biofilms are much more resistant to destruction than planktonic microorganisms. Although the mechanisms of this resistance are poorly understood, EPS are likely to play a role. In addition, biofilm bacteria are substantially resistant to surfactants, biocides and antibiotics. Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in food poisoning or infections. It has also been proposed that biofilms could allow the multiplication of microbial pathogens stochasticly present in freshwater, as well as providing a mechanism for bioaccumulation of toxic substances. As a result, microbial biofilms constitute major industrial and medical concerns. These concerns are now being realized in the dental profession.

Dentists, dental surgeons and dental hygienists and their patients are well aware of the importance of meticulously sterilizing and disinfecting dental instruments. Indeed, since dental instruments are used directly in a patient's

mouth, sometimes for invasive or surgical procedures, it is of paramount importance to minimize the presence of microorganisms carried by dental instruments. The microorganisms can range from relatively harmless bacteria to dangerous pathogens. Consequently, efforts are deployed to remove microorganisms from dental instruments and from the fresh water lines feeding dental instruments such as air/water seringes, high speed turbines, and ultrasonic scalers, or from saliva evacuation lines. For most hand held dental instruments, thermal sterilization remains one of the best methods for eradicating microorganism. However, thermal sterilization is obviously not practical for the decontaminating of fresh water lines which remain to this date difficult to rid of microorganisms.

It is well known in the dental profession that small diameter pipes carrying fresh water are contaminated by bacteria and other microorganisms contained in the water flowing through them (Barbeau *et al.* 1996). Some of the microorganisms inevitably adhere to the inner walls of the lines and accumulate together with microscopic sediments or other substances into what is commonly known as a biofilm (Barbeau *et al.* 1997). The biofilm quickly and tenaciously coats the inner walls of the lines and becomes a reservoir for the proliferation of microorganisms. Bacterial populations will rapidly reach alarming levels which will also be found in the water discharge from the dental instruments connected to the fresh water line. For example, the average bacteria count in the water discharge of dental instruments is known to be of approximately 200,000 colony forming units per milliliter (cfu/ml) and in some extreme cases can reach 10,000,000 cfu/ml (Barbeau *et al.* 1996).

Jacquelin *et al.* (Path. Biol. 42(5): 425-431 (1994)) disclose compositions comprising a detergent such as sodium dodecyl sulfate (SDS) or sodium deoxycholate (SDC) and a phenolic disinfectant. The solutions are not efficient to remove and/or destroy biofilms as seen from the photographs of Figure 1 and from the concluding remarks of this reference.

Whittaker *et al.* (Appl. and Env Microbiol. 43(3): 395-403 (1984)) disclose a plurality of compositions tested for their cleaning/disinfecting properties against micoorganisms. Their best composition was SDS/urea, which was efficient on chlorine-treated osmosis membranes after 11 days of

treatment, which time is far from being a practical cleaning/disinfecting time for dentistry.

European patent publication 109 279 describes a solution comprising a plurality of essential ingredients for sterilizing surgical apparatuses. Although this reference suggests that biofilm decontamination is contemplated, there is no demonstration whatsoever on that specific issue. Moreover, there is no teaching of any subset of combined ingredients which would be capable by itself to remove the biofilm, and optionally, to kill the embedded bacteria.

A commercially available mouthwash sold under the trademark PLAX which comprises SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%, supposedly helps in removing dental plaque prior to tooth brushing. The efficacy of this solution against biofilms in general is however doubtful given the short time of contact within which dental plaque is to be removed, even when tooth brushing follows.

Patent publication WO 96/20737, assigned to the present proprietor, describes compositions capable of cleaning and disinfecting biofilm-coated surfaces. These compositions comprise SDS 1% - 2%, hydrogen peroxide 5%, EDTA 1%, mandelic and lactic acids in individual 1% concentration or in combined 2% concentration (mandelic acid being a bactericide). They further describe sub-compositions comprising the same concentrations of SDS/hydrogen peroxide/EDTA and SDS/acids. There is no teaching in these publications of compositions which would be different therefrom and still equivalent thereto, and there is no teaching of how specific components attack the integrity of the biofilms e.g. there is no mechanism of action proposed which would lead to establish a generic class of components useful for the purpose of removing biofilms with high efficacy.

Another mouthwash has an excellent bactericide activity, that is CEPACOL™, which comprises a low concentration of cetylpyridinium chloride and ethanol. CEPACOL does not remove biofilms but is very efficient against planktonic bacteria. Cetylpyridium chloride (CPC) has been shown as one possible components in the solutions disclosed in WO 96/20737, and it also was used at a low concentration (0.1%). CPC being also a detergent, we hypothesized it could be used at higher concentrations to replace SDS and hydrogen peroxide.

USP 4,961,923 describes an irrigant to be used in a dental apparatus, which comprises water, ethanol, hydrogenated starch hydrolysate and a surfactant. The surfactant is preferably polysorbate 80. The irrigant may further comprise a disinfectant like cetylpyridinium chloride or other mouth wash-composing ingredients. This patent aims at dislodging bacteria from a dental pocket with mechanical aid and not at removing well-established biofilms and this, without necessitating any mechanical aid. A composition comprising organic salts or salt forming acids in combination with detergents is not taught in this patent. Further, this document teaches the use of ethanol and surfactants solely for the purpose of dissolving the components, and not for their biofilm dislodging or bactericidal properties.

USP 3,941,696 describes the use of cetylpyridinium chloride as a disinfectant. There is no teaching of a composition which would be capable of dislodging established biofilms.

USP 5,948,390 describes a mouthwash comprising about 0.01%-1% by weight of hydrated uncomplexed zinc cations, about 0.01%-4% by weight of fully or partially protonated citrate moieties; about 0.01%-2% by weight of cetylpyridinium moieties, and an orally acceptable vehicle. This mouthwash composition is formulated at pHs of 3.0 to 4.5. This patent focuses on the addition of a zinc component in a CPC-comprising mouthwash. This patent aims at adding zinc for its anti-microbial and deodorizing properties, while solving the problem of zinc unpleasant and astringent taste. The addition of protonated citrates appears to prevent zinc complex formation, at pH of 3.0 to 4.5.

There still remains a need for compositions for cleaning biofilm-coated surfaces which will effectively dislodge a biofilm and optionally kill the microorganism flora in the dislodged biofilm, these compositions being adapted upon a variety of industrial uses and needs.

SUMMARY OF THE INVENTION

Against all expectations and documented evidence, the present inventors found that effective removal of biofilm may be achieved, using a solution minimally comprising a detergent and acids which, at the working pH, form salts in a substantial proportion. These two components by themselves are sufficient to remove well-established biofilms in a period of

time varying from within 1 hour to an indefinite time, more preferably between about 1 hour and 18 hours.

When destruction of microorganisms is a concern, particularly in the medical or dental professions, a bactericide must be added to the solution. The bactericide contacts the surface rid of biofilm and wherein residual microorganisms retained on the surface will be killed. Preferably, the disinfecting and cleaning actions are allowed to occur concurrently.

In accordance with the present invention is provided a solution for dislodging a biofilm from a surface, which comprises an effective dislodging amount of a detergent and an effective dislodging amount of a salt or of an acid which forms a salt at a working pH value, or both, said salt being capable of displacing divalent cations present in the structure of the biofilm with the proviso that the composition is neither a mixture of SDS 1 % - 2 % and EDTA 1%, a mixture of SDS 1% - 2% and mandelic and lactic acids, each at an individual concentration of 1% or in a combined concentration of 2%, a mixture of SDS 0.25%, sodium salicylate 0.2% and sodium benzoate 2% (PLAX), nor a mixture of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA (EP 109 279), all percentages representing final weight per volume concentrations.

It is another object of this invention to provide a composition for dislodging and destroying a biofilm, which further comprises a bactericidal amount of a bactericide.

In preferred embodiments, the detergent is SDS in a concentration of at least about 0.1 % or any detergent having a biofilm dislodging potency substantially equivalent thereto. The acid is mandelic acid in a concentration of at least about 0.1 % at a working pH value (pH 5 is one example), or a mandelate salt, or any acid or salt having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH. For example, the salt or acid may interestingly be an EDTA salt or acid in a concentration of at least about 0.25 % at a working pH value. At pH 5, EDTA acid forms EDTA salt and is performing when combined to SDS, with or without any other acid, although better results were obtained with another acid.

In more preferred embodiments, the acid is selected from the group consisting of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic,

fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic acids, alanine, serine, tryptophan, tyrosine, bicine, tricine and glycine. When a bactericidal activity is needed, a bactericide such as hydrogen peroxide or any bactericide having a bactericidal potency substantially equivalent thereto may be added. Other bactericides like phenol derivatives or sodium hypochlorite are examples of good bactericides. They have been used in concentrations of at least 0.1% and 0.5 %, respectively. In even more preferred embodiments, the composition further comprises biofilm dislodging enhancer agents such as chaotropic agents or calcium chelators.

A calcium chelator such as EDTA, preferably in a salt form, in a concentration of at least about 0.25 % or any calcium chelator having a chelating potency substantially equivalent thereto may be added.

A chaotropic agent such as SDS in a concentration of at least about 0.1 % or any chaotropic agent having a chaotropic potency substantially equivalent thereto may also be added.

In more preferred embodiments, the compositions comprise at least about 0.1% SDS, at least about 0.1% acid, at least about 0.25% EDTA, the acid being selected from the group consisting of 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, aspartic, phosphoric, pyruvic, chloroacetic acids and alanine.

In a mostly preferred embodiment, the compositions comprise at least about 0.1% but less than 1% SDS, about 0.1% - 2% acid, and at least about 0.25% but less than 1% EDTA, the acid being mandelic acid or any other of 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, aspartic, phosphoric, pyruvic, chloroacetic acids and alanine.

The highest concentrations confer a strength to the composition such as it is effective within one hour. The lowest concentrations confer a good performance within 18 hours.

Good bactericides comprise mandelic acid 1%, hydrogen peroxide about 5%, or phenol derivatives at least about 0.1%, or sodium hypochlorite at least about 0.5% These bactericides are tuberculocides e.g. they are

active against *Mycobacterium* spp. which are resistant to a large panel of bactericides.

We have further found that cetylpyrinidium moieties such as chloride or bromide (CPC or CPB) were very good biofilm dislodgers and bactericides at a concentration higher than 0.5%, when combined to EDTA 1% and a salt or a salt-forming acid 1%, at pH of 7.5. The tuberculocide activity was very good with mandelic acid 1%.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Each component tested in this application will be given an abbreviated name, which complete definition is as follows:

SDS	Sodium dodecyl sulfate
EDTA	Ethylenediamine tetraacetic acid
H_2O_2	Hydrogen peroxide
CPC	Cetylpyridinium chloride
CPB	Cetylpyridinium bromide
Tween 20	Polyoxyethylene sorbitan monolaurate
SCS	Sodium cocoyl sarcosinate
SLS	Sodium lauryl sarcosinate
SDDD	Sodium n-decyl diphenylether disulfonate
HEEDTA	N-(hydroxyethyl)ethylenediamine triacetic acid
DTPA	Diethylenetriamine pentaacetic acid

Starting from the solutions already described in the patent publication WO 96/20737 assigned to the same proprietor, comprising SDS 1% - 2%/mixture of mandelic and lactic acids 2%/EDTA 1%/hydrogen peroxide 5%, we first replaced mandelic and lactic acids with a plurality of acids used individually in 1% concentration (w/v), the pH of the working solution being brought to 5.0. We further tried different components with hope to find equivalents for each other essential and non-essential ingredients of the composition.

The compositions were allowed to contact biofilms for 1 and 18 hours to evaluate their cleaning and disinfecting efficacy.

Testing the disinfectant.

Optical and scanning electron microscopy.

Two-cm long pieces of dental unit waterlines tubings were used. These tubings were taken from functional dental units installed at the faculty of dentistry of the University of Montreal. Our previous studies (1992-1996) have shown that the lumen of these tubings is covered with mature biofilms. The pieces were sectioned longitudinally with a sterile scalpel blade to expose the biofilm. Another series of tubings was left untouched. Sections of tubings were placed in sterile 5ml disposable test tubes containing solutions to be tested.

After 1 or 18 hours without agitation at room temperature, tubings were rinsed three times with sterile water. Examination was done first with a binocular microscope at a magnification of 40X. Data were recorded on a arbitrary scale by two different examiners and noted as from 4+(same as control) to 0 (no biofilm).

Selected pieces of tubings were processed for electron microscopy.

Scanning electron microscopy (SEM)

For SEM observations, following fixation, post-fixation and dehydration, samples were critical point dried with carbon dioxide in a Balzers CPD 030 Critical Point Dryer (Balzers, Furstentum, Liechtenstein), then mounted with a conductive carbon paint on aluminum stubs and sputtered with gold, or carbon-evaporated in a Bal-Tec MED 020 High Vacuum Coating System (Bal-Tec Products Inc., Middlebury, CT, USA). The interior of tubing segments was examined with a field emission JEOL JSM 6300F SEM operated at an accelerating voltage of 15 kV. The gold-coated specimens were used for SEI while the carbon-coated ones were visualized by BEI.

Antibacterial activity.

To test the antibacterial activity, we used the same setup as above. After the 1 or 18 hour incubation, tubings were rinsed with sterile water. Pieces of tubings were dropped in sterile test tubes containing 4 ml of R2Am medium. Test tubes were capped and incubated for 7 days at room temperature. These conditions proved to be best for the growth of the majority of dental unit waterlines bacteria. Data were recorded as presence or absence of growth with a spectrophotometer at λ=590 nm.

We have isolated 30 strains of DUWL bacteria among which, *P. aeruginosa, P. putida, M. mesophilicum, A. calcoaceticus, P. fluorescens* were the most frequent species. We tested these strains individually in the disinfectant. Pure culture of bacteria in liquid R2Am broth were used.

***In situ* testing.**

The disinfectant was tested in the ACCM© prototype in a closed room at the Faculty. The ACCM was installed on a A-dec dental unit by one of our technicians. Before the study, water samples were taken for bacterial counts, and a two-cm piece of the air/water syringe hose was taken for SEM. The lines were filled with disinfectant containing alizarin green as an indicator and the setup was left unused overnight. The next day, the disinfectant was drained until no coloration was seen. Draining was done for an extra 2-min and a 4-ml water sample was taken for bacterial counts. Another 2-cm piece of tubing was sectioned for SEM. A second sample was taken at the end of the day and the lines were filled with disinfectant for 18 hours. This routine was repeated over a period of one month. In some experiments, the disinfectant was left to react for 1 hour in lieu of 18 hours.

Collection and plating of water samples.

All the water samples were vigorously agitated with a vortex for 15 seconds. The plating was done by inoculating Petri dishes with 100 µl of a 1:10, 1:100 and 1:1000 dilution in duplicate, or by using an automatic spiral plating system (Meyer Service & Supply, Ontario, Canada) after a tenfold dilution of the sample. The enumeration was done using a magnifying glass and a counting grid.

Control samples (20 ml) were obtained from nearest taps in each clinic and at the source upstream to the connection to the dental unit in selected units. These samples were filtered through a 25 mm polycarbonate filter (0.22 µm) (Millipore, Montréal, Canada) using a sterile syringe and a filter holder (Millipore). The filters were then placed on the surface of the culture medium in a Petri dish and incubated.

Newly installed dental units (Kavo, Germany) at the dental school were also sampled just before their first clinical use with the same sampling technique.

Culture conditions.

A modification of the medium of Reasoner (termed R2Am) was used. The composition is as follows: starch 0.5g, yeast extract 0.5g, trypticase peptone 0.5g, dextrose 0.5g, K_2HPO_4 0.3g, $MgSO_4$ 0.05g, succinate 0.25g, casamino acids 0.5g, agar 7.5g, and distilled water to 1L. Tryptone soy agar and Sheep blood agar (Difco, Montréal, Canada) were also used. Bacteria were cultivated in aerobiosis and anaerobiosis (10% CO_2, 10% H_2 and 80% N_2, anaerobic cabinet: Forma Scientific, Montréal, Canada) for the determination of their dependency on oxygen and at 25°C and 37°C over time between 24 and 480 hours.

Towards the establishment of a quantitative assay

A more quantitative assay has been built as follows: water samples were collected from dental units and were two-fold diluted with a R2Am medium (which composition per liter is:)

1 g soluble starch (Sigma # S-9765)
1 g yeast extract (BBL 11929)
1 g trypticase-peptone (BBL 11921)
1 g dextrose (Difco Laboratories # 0155-17-4
0.1 g magnesium sulphate anhydre (Sigma # M-7506)
0.5 g succinic acid (Sigma # S-9637)
1 g casamino acid (Difco Laboratories (0230-01)
0.6 g potassium phosphate diabasic (Sigma # P-3786).

The medium was autoclaved for 20 minutes at 121 °C.

250 µl of the diluted mixture were distributed in each well of a 96-well plate. The plate was incubated at ambient temperature for 2 days. 100 µl were withdrawn from each well, and daily replaced with 100 µl fresh medium, during four days. Biofilms representative of those growing and coating the dental water line thus formed in the wells. The solutions to be tested were left in contact with the biofilms for 1 to 18 hours. The solutions were withdrawn and the wells were rinsed. The presence of bacteria remaining in the wells, if any, was revealed with violet crystal dye coloration for one hour. The dye was withdrawn and the wells were rinsed. The dye extracted in 300 µl of a mixture of 40% methanol (v/v) and 10% acetic acid (v/v). The color intensity was read by spectrophotometry at 570 mm on 100 µL aliquots. The absorbance correlates with the residual quantity of biofilm.

Acid Substitutions:

We first substituted a plurality of acids for mandelic and lactic acids. All these acids were used in 1% (w/v) final concentration. Results that have been obtained with the semi-quantitative assay are shown in Table 1.

Scale evaluation :

- : no biofilm and ++++: no significant removal of film.

TABLE 1: ACIDS SUBSTITUTING FOR MANDELIC OR LACTIC ACIDS

DESCRIPTION					RESULTS AFTER DISINFECTION		
Selected Acid (1%)	H_2O_2	EDTA	SDS	pH	1 hour	18 hours	Growth
Mandelic Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Lactic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
D-Tartric acid	5%	1%	1%	5,0 (NaOH 10%)	++	++	-
Citric Acid	5%	1%	1%	5,0 (NaOH 10%)	+++	+	-
Oxalic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	+	-
Oxamic Acid	5%	1%	1%	5,0 (NaOH 10%)	+++	+	-
Sulfamic Acid	5%	1%	1%	5,0 (NaOH 10%)	+++	++	
Malonic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	++	-
Malic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Dichloroacetic Acid	5%	1%	1%	4,99 (AcOH)	+	+	-
2-Ketoglutaric Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Maleic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Succinic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Acetic Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Phenylacetic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	+	-
R-Chloromandelic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-

DESCRIPTION					RESULTS AFTER DISINFECTION		
Selected Acid (1%)	H_2O_2	EDTA	SDS	pH	1 hour	18 hours	Growth
L-Serine	5%	1%	1%	5,0 (AcOH)	+	+	-
D-Phenylalanine	5%	1%	1%	5,0 (HCl 10%)	++	++	-
Glutamic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
R-2-Phenylglycine	5%	1%	1%	5,0 (AcOH)	++	++	-
Glycine	5%	1%	1%	5,0 (AcOH)	++	++	-
Benzilic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	+	-
Iminodiacetic Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Nitrilotriacetic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Tricine	5%	1%	1%	5,0 (AcOH)	+++	+	-
Bicine	5%	1%	1%	5,0 (AcOH)	++	+	-
Mucic Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
L-Aspartic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	-	-
L-Ascorbic Acid	5%	1%	1%	5,0 (NaOH 10%)	++++	+++	-
Phosphoric Acid (H_3PO_4)	5%	1%	1%	5,0 (NaOH 10%)	++	-	-
Boric Acid ($B(OH)_3$)	5%	1%	1%	4,99 (AcOH)	+	+	-
Pyruvic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	-	-
Glycolic Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Adipic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-

DESCRIPTION					RESULTS AFTER DISINFECTION		
Selected Acid (1%)	H_2O_2	EDTA	SDS	pH	1 hour	18 hours	Growth
Formic Acid	5%	1%	1%	5,0 (NaOH 10%)	+++	+	-
Glucuronic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Salicylic Acid	5%	1%	1%	5,0 (NaOH 10%)	++	+	-
Benzoic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Benzoylformic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Chloroacetic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	-	-
Phthalic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
Fumaric Acid	5%	1%	1%	5,0 (NaOH 10%)	-	-	-
Ketopimelic Acid	5%	1%	1%	5,0 (NaOH 10%)	+	+	-
L-Tryptophan	5%	1%	1%	5,0 (AcOH)	+	+	-
DL-Alanine	5%	1%	1%	5,0 (AcOH)	+	-	-
DL-Tyrosine	5%	1%	1%	5,0 (AcOH)	+	+	-

To our surprise, all the tested acids manifested a capacity to dislodge bioflims, leaving the biofilm constituents free for the bactericidal activity of hydrogen peroxide. Some acids conferred a slow but nevertheless significant tendency of the solution to dislodge biofilms with time, while the others were more rapid in this respect. We have selected cleaning solutions capable of performing at practical times: 1 hour or less, and overnight (about 12 - 18 hours). From the above list, seven acids were performing within less than 1 hour: mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic and fumaric acids. To this restricted list of very performing acids can be added those acids performing between 1 and 18 hours: lactic, aspartic, phosphoric, pyruvic, chloroacetic acids and alanine. Since the above results are of semi-quantitative nature only, we add to the list of acceptably performing acids: oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoylformic, phthalic, ketopimilic acids as well as serine, tryptophan, tyrosine, bicine, tricine and glycine, because they all led to sufficient decontamination within the specified times. All the other acids can be used when time is not a major concern or when mechanical brushing is used to accelerate removal of biofilm. It is worthwhile noting the isomeric form of an acid does not appear to influence its dislodging capabilities.

Taking the results of acid substitution altogether, it appeared clear that the common point between the acids was that they may form salts at the working pH of 5.0.

These observations led us to appreciate why the sub-compositions already published by the present assignee minimally comprising SDS/acids were performing cleaning and disinfecting solutions. The presence of the detergent and salt forming acids is most probably responsible for breaking the integrity and dislodging the biofilm. Since mandelic acid is also a bactericide, the combination SDS/acids was also an efficient detergent/ disinfectant solution.

Further, the other sub-compositions disclosed in the same patent publications minimally comprising SDS/EDTA/hydrogen peroxide, which were also performing sub-combinations revealed that EDTA used as a salt was

capable of complementing SDS in the cleaning component of the solution. Hydrogen peroxide had the role of the bactericide.

It is therefore an object of this invention to provide a minimal cleaning solution comprising a detergent and a salt forming acid (direct addition of salts in the solution is also an option). The bactericides are added in the solutions, when a bactericidal complement is desired. Any bactericide may be added to the above cleaning solution, which would have for effect to confer an additional disinfecting action thereto, which action is greatly facilitated by the dislodging action of the cleaning ingredients: detergent and salts. Amongst the bactericides tested, sodium hypochlorite, phenol derivatives and hydrogen peroxide showed a broad host killing activity, even against *Mycobacterium* sp. known for their high level of resistance towards bactericides. Mandelic acid has a dual role as a salt forming acid and as a bactericide. Povidone-iodine was also tested and had a significant efficiency when combined to the detergent Tween 20™. Cetylpyridinium chloride and bromide have a dual role as a detergent and a bactericide. All the above bactericides are non limitative examples of bactericides.

Equivalents:

The above Table I shows that many acids are equivalent to mandelic and lactic acids.

Different types of detergents have been tried, a cationic one (cetylpyridinium chloride (CPC) and bromide (CPB), also bactericides), as well as hexadecyltrimethylammonium bromide (CTAB) and benzalkonium chloride, non-ionic ones (Tween 20™, MEGA-8 (octanoyl-methyl-glucamide) (O-3129), MYRJ-45 (polyoxyethylene ester-8-stearate) (P-3315), Polidocanol (polyoxythylene-9-lauryl ether) (P-9641), Tergitol NP-40 (polyglycol ether) (NP-40), Triton X-100 (octylphenoxypolyethoxyethanol), Tween-85 (polyoxyethylene sorbitan trioleate) (P-4634), Tyloxapol (polyoxyethylene ether) (T-0307) and povidone-iodine, also a bactericide), anionic ones (SDS, SCS and SDDD, alginic acid high and low viscosity (A-7128), Cholic acid (C-1254) and Lithium docecyl sulfate) and zwitterionic ones (CHAPSO (C-3649)(3-((3-cholamidopropyl)dimethyl-ammonio)-2hydroxy-1-propanesulfonate) and SB3-10 (alkyl-dimethylammono-propane sulfonate)). These detergents were capable of dislodging biofilms to various extents. SDS, CPC and CPB were the preferred ones. SDS achieved very good

activity even at a concentration as low as 0.15% after one hour, and a perfect efficiency at the same concentration after 18 hours. It is worthwhile noting that the solution tested with 0.15% SDS also contained 6% hydrogen peroxide, and low amounts of HEEDTA (acid; 0.3%), acetic acid (0.1%) and zinc sulfate heptahydrate (0.1%). This solution corresponds to the best one described in EP 109 279. The pH of the solution was adjusted from 2.42 to pH 5, which entails of salt formation. Our minimal solutions would comprise a detergent and salts. As a first optional goal, they would also comprise a bactericide. CPC and CPB fulfilling both detergent and bactericide sought activities, they became our best candidates as detergents/bactericides.

Further, the commercial mouthwash PLAX™, comprising 0.25% SDS, 2% sodium benzoate and 2% sodium salicylate (pH 7.35) showed good biofilm removal, although not perfect, after 18 hours of contact. The acceptable performance of that solution confirms that salts only may be used in lieu of acids.

The above results show that the concentration of detergent and salts may be quite low if the time of contacting is longer (for example overnight), while higher concentrations confer more strength and decrease the time necessary for dislodging biofilms (within 1 hour for example).

Enhancers:

As mentioned above, we already described sub-combinations which were as efficient as the complete combination SDS 1% - 2 %/EDTA 1%/ hydrogen peroxide 5%/mandelic and lactic acids 2%. These sub-combinations comprise the ingredients SDS/EDTA/hydrogen peroxide and SDS/acids. In both cases, sub-compositions comprise the ingredients detergent and bactericide. What was not explained at the time was why EDTA was essential to the first sub-composition to perform well. The above results provide such an explanation: EDTA salts greatly improve the dislodging or cleaning capacity of the solutions. The presence of EDTA is no longer deemed necessary to present cleaning solutions, since EDTA may be replaced by other salts or salt forming acids. EDTA is rather considered as an activity enhancer, because this compound is also a good divalent ion chelator, and as such, it may help in withdrawing Ca^{2+} ions from the polysaccharide biofilm matrix, leading to a faster dismantlement thereof.

SDS was one of the preferred detergents and it is further worthwhile noting that this detergent is also a chaotropic agent. It is therefore contemplated that a chaotropic agent may be optionally added to increase the biofilm dislodging strength of the solution. Such chaotropic agents include but are not limited to SDS, urea and guanidine. The chaotropic agent is also considered as an optional activity enhancer.

Additives:

Should the detergent used in the composition produce foam, it might be desirable to add an anti-foamer. Also, a dye might be added to the compositions of this invention for easy monitoring of the extent of rinsing. Further, flavors or scents may be added to provide a pleasant taste or smell to surfaces to be cleaned.

Fresh water lines supplying dental instruments are of a very small diameter, which excludes the possibility of scrubbing. This would not be the case for dentures, surfaces or tubings of larger diameter. The compositions of the present invention have the advantage of showing efficient decontamination in the complete absence of scrubbing in a convenient time of decontamination. The present invention is not only useful for dental instruments or protheses. It will become obvious that it is intended for other applications, e.g. cleaning or decontaminating any type of tubing or container on the surface of which microorganisms are adsorbed and form a biofilm. In such other applications, scrubbing or any other mechanical aid is not at all excluded. Should these compositions be used in pipes of a larger diameter and length, for example, wherein a non-cost effective large volume of cleaning solution would be needed to fill the pipes, it is possible that a mechanical action would help in the action of the solution. A mechanical aid, when envisaged, would help in reducing the duration of cleaning and/or in spreading the cleaning solution on a surface. It is further not excluded to add a vehicle allowing the cleaning solution to stay in contact with the surface to be decontaminated as long as possible. This is referred to as an adhesion enhancer. Some or all of cleaning solution components might be delivered in separate vials, in concentrated or in solid forms, to be admixed in the final reconstitution water volume. This could reduce the handling and storage of large volumes of decontaminating solutions.

Minimal and Optimal Effective concentrations of components

Detergents:

SDS has been tried at a final concentration of 0.15% (w/v) and did work well within one hour. So, SDS certainly can be as low as about 0.1% when the duration of treatment may last about one hour or more. The most preferred SDS concentration was 1% which achieved a perfect cleaning efficacy within one hour. Any detergent at a concentration as potent as at least about 0.1% SDS is within the scope of this invention. For example, the following detergents and concentrations have been tried with success:

- SDDD 0.015% - 1%,
- SCS 0.3% - 1%,
- Tween 20™ 4%, and
- CPC 0.5%-1%, and
- CPB 0.5%-1%.

So, detergents of all classes: non-ionic, anionic and cationic, have been all successful in removing biofilms, and this invention should not be limited to the tested five detergents.

Salt-forming acids:

Mandelic acid has been tried with success in a concentration extending from 1% to 10%. Besides that, acetic acid has been tried in a range of concentrations 0.1% to 1% and was very efficient. Further, a plurality of acids (1%) may substitute for mandelic acid 1% (see Table 1). It is therefore contemplated that acids can be used in a minimal concentration of about 0.1% at a salt-forming pH. Preferred acid concentration is 1% for rapidly acting solutions, with reference to mandelic acid. Any acid capable of forming salts at the working pH, in concentrations equipotent to at least about 0.1% mandelic acid, depending on the desired contacting time, is within the scope of this invention. Although a pH of about 5 has been tested, it is readily apparent to a skilled reader that the pH of the solutions is not restricted to that value. Indeed, when CPC was the used detergent, a solution having a basic pH (namely pH 7.5) was preferred.

Cleaning Enhancers:

Chelators:

Tetrasodium EDTA (0.25% - 1%) has been tried with a certain degree of success against biofilms. Any chelator in a concentration equipotent to the above concentrations of EDTA is within the scope of this invention. It is worthwhile noting that HEEDTA has been used in the acid form (0.3%) and was good when another salt forming acid: acetic acid, was at a concentration of 0.1% to 1% and when the pH was brought from 2.42 to 5.0. So, chelator salts can be used or chelator acid precursors can be used in salt forming conditions. It is recalled that the chelator is an optional component; it is used to increase the cleaning strength of the solution. Its function is mainly to capture divalent ions such as Ca^{2+} which are involved in EPS integrity.

Chaotropic agents:

SDS has a dual action as a detergent and a chaotropic agent. Since a plurality of non-chaotropic detergents may substitute for SDS, the chaotropic activity is not considered essential to the claimed compositions. However, since SDS was one of the preferred detergents, it is contemplated that a chaotropic agent may be useful, as an optional component, in increasing the cleaning strength of the solution. Any chaotropic agent having the potency of in a concentration of at least about 0.1% SDS is within the scope of this invention.

Bactericides:

When it is desirable to complete the cleaning solution with a bactericidal activity, especially in the medical field, a bactericide can be added in an effective concentration. It is recalled that bactericides alone are less effective against biofilms than against planktonic micoorganisms. However, when bactericides are combined to a detergent/salt solution, or contacted with surfaces thereafter, they are capable of killing microorganisms which are retrieved as planktonic organisms and no longer organized as a biofilm, due to the detergent/acid/salt effect. Povidone-iodine 10%, mandelic acid 1%, sodium benzoate/salicylate 2%/.2%, hydrogen peroxide 5%, sodium hypochlorite 0.5%, phenol 0.1% and CPC 0.1%-1% with or without ethanol have all been tried with success; which indicates that any bactericide may be added in the cleaning solution in so far as the selected bactericide has a killing activity against the populations of microorganisms to eliminate.

Amongst the above-listed bactericides, we have preferred mandelic acid, hydrogen peroxide, sodium hypochlorite, phenol, CPC, and CPB because these bactericides qualify as tuberculocides; they are efficient against highly resistant *Mycobacterium* species and they have a large spectrum of efficiency against microorganisms. Table 1 shows that hydrogen peroxide really killed the bacteria, which translated into a quasi-total absence of growth after treatment.

Dismantling the exopolysaccharides (EPS) present in the biofilms:

EPS exist in more or less ordered forms in natural environment. Many bacterial EPS appear to adopt a double helicoidal configuration and the association of the double helices is facilitated by ions (such as Ca^{2+}) and by water molecules. The physical properties of EPS and hence of biofilms may be influenced by the presence of free anionic groups (uronic acids, phosphate groups, pyruvate ketals or succinyl half esters). Hydrogen bonding involving exposed hydroxyl groups can also be significant. Localized hydrophobic regions may also exert influence. Therefore interacting with the ions involved in the maintenance of cohesive biofilms is a target for the dismantlement thereof. It has been suggested that excess Na^+ may exchange with Ca^{2+} and that local proton gradients may convert the salt form of the EPS to the proton form, again altering its properties. (I.W. Sutherland in "Biofilms, Community Interactions and Control" ed. J. Wim-Penny, P. Handley, P. Gilbert, H. Lappin-Scott and M. Jones. Third Meeting of the British Biolfim Club, Powys, Sept. 1997).

Even if it may have been envisaged that changes in the ionic environment of a biofilm may alter its integrity, no one has ever come up with satisfyingly performing solutions, or when such solutions exist, there has been no teaching of using them for removing biofilms and this, without any mechanical aid. The present invention relates to compositions and methods minimally comprising or making use of salts or salt-forming acids and a detergent. Preferably, a salt-forming acid is used to create an equilibrium between acids and salts, both of which being involved in attacking the biofilm components. The detergent appears to have a synergistic effect in solubilizing the components and in exposing EPS sub-layers which, in turn, become attackable by acids and salts. The present ingredients when combined are very efficient and achieve complete removal of biofilms.

Bactericidal agents, when added, become efficient against biofilms, because the biofilm becomes disorganized and is no longer impermeable to the anti-microbials.

Towards making a cost-effective solution:

Using H_2O_2 in water lines is not ideal because of a consequent oxygen pressure building therein. As a promising alternative to hydrogen peroxide, we tried CPC and CPB, which are good and equivalent detergents as well as good bactericides. CPC has been preferred over CPB, because of its lower cost. CPC is a bactericidal detergent normally used at concentrations ranging from 0.01% to 2% (w/w). The best biofilm dislodging activity was found at concentrations higher than about 0.5%. Amongst the acids listed above, four of them did not quantitatively decrease the biofilm dislodging capacity of a solution also comprising CPC 0.5%, EDTA 1% at pH 7.5; these acids where glycolic, fumaric, citric and phosphoric acids at 1% concentration. The biofilm dislodging efficiency of these solutions was very good. It is believed that other components could be admixed thereto to bring this efficiency to perfect. The bactericidal activity against *Mycobacterium gordonae* of a solution comprising CPC 0.5%, EDTA 1%, mandelic acid 1% (all w/v), at pH 7.5, was compared to CEPACOL™, a positive bactericidal control. A growth diminution of 85% has been observed with our solution, while CEPACOL™ inhibited the same by almost 100%. CEPACOL™ comprises CPC and ethanol. CEPACOL™ is a good bactericide but not a good biofilm dislodger. We believe that anyone of our solutions comprising CPC 0.5%, EDTA 1% and any of glycolic, fumaric, citric, phosphoric acids 1%, at a salt-forming pH, could be complemented with ethanol as well as with anyone of the above-listed enhancers. For example, the biofilm dislodging capacity is very good with SDS which is a detergent and a chaotropic agent. It is not excluded that SDS could be added to CPC, both of them being combined for their detergent action and for their chaotropic and bactericidal actions, respectively. The concentrations of SDS and CPC could even be reduced if such a reduction is not detrimental to the biofilm dislodging efficacy. Ethanol may be added for its enhancing efficiency on the tuberculocide activity of our solutions. Any buffering agent necessary to bring the pH at a proper value can be added.

One of our goals is further to provide the most cost effective solution for biofilm removal (and destruction). It would be advantageous for all or the

majority of the components of our solutions to be in a solid form, with or without buffering agents, with or without additional bactericidal components. Using salts of organic acids and solid detergents can be advantageous because they may have a longer shelf life, they require less storage space, and they all be extemporaneously admixed to a determined volume of tap water. Ethanol may be optionally provided in a separate container if desirable. Alternatively, pellets can be provided in lieu of a powder. For the purpose of providing a denture cleaner, for example, a rapidly disintegrating pellet comprising all the solid ingredients and proper excipient can be produced.

It is further not excluded to add adhesion enhancers at more or less elevated concentrations. When the compositions are to be used in water lines of low cross-section the adhesion enhancers which frequently increase the solution viscosity should be in a rather low concentration. Adhesion enhancers such as glycerin or Pluronics would be added to increase the propensity of the components to come in close contact with the biofilm, when desirable.

Although the present invention has been described herein above by way of preferred embodiment thereof, these embodiments can be modified at will without departing from the spirit and the nature of the subject invention. These modifications are within the scope of this invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A composition for removing a biofilm from a surface, which comprises an effective dislodging amount of a detergent and an effective dislodging amount of an acid or a salt of an acid, said salt being capable of displacing divalent cations present in the structure of said biofilm, with the proviso that said composition is neither a mixture achieving an aqueous final concentration of SDS 1 % - 2 % and EDTA 1%, of SDS 1% - 2% and mandelic and lactic acids, each at an individual concentration of 1% or in a combined concentration of 2%, of SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%, nor a mixture of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA, all percentages representing weight per volume concentrations.

2. A composition as defined in claim 1, further comprising a bactericidal amount of a bactericide.

3. A composition as defined in claim 1, wherein said detergent is SDS, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any detergent having a biofilm dislodging potency substantially equivalent thereto.

4. A composition as defined in claim 3, wherein said equivalent detergent is CPC or CPB at a concentration of at least about 0.5%.

5. A composition as defined in claim 2, wherein said detergent is SDS, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any detergent having a biofilm dislodging potency substantially equivalent thereto.

6. A composition as defined in claim 5, wherein said equivalent detergent is CPC or CPB at a concentration of at least about 0.5%.

7. A composition as defined in claim 1, wherein said acid is mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

8. A composition as defined in claim 2, wherein said acid is mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

9. A composition as defined in claim 1, wherein said salt or acid is an EDTA salt or acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any salt or acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

10. A composition as defined in claim 2, wherein said salt or acid is an EDTA salt or acid which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any salt or acid having a biofilm dislodging potency substantially equivalent thereto at a suitable working pH value.

11. A composition as defined in claim 1, wherein said salt or acid is sodium mandelate or mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration range of at least about 0.1 % at a working pH value or any salt having a biofilm dislodging potency substantially equivalent thereto.

12. A composition as defined in claim 2, wherein said salt or acid is sodium mandelate or mandelic acid which achieves, once reconstituted in an aqueous solution, a concentration range of at least about 0.1 % at a working pH value or any salt having a biofilm dislodging potency substantially equivalent thereto.

13. A composition as defined in claim 1, wherein said acid is one or more of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, mandelic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine.

14. A composition as defined in claim 2, wherein said acid is one or more of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, mandelic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine.

15. A composition as defined in claim 2, wherein said bactericide is hydrogen peroxide or any bactericide having a bactericidal potency and host spectrum substantially equivalent thereto.

16. A composition as defined in claim 15, wherein said equivalent bactericide is mandelic acid, phenol, sodium hypochlorite, CPC or CPB.

17. A composition as defined in claim 16, wherein mandelic acid or salt, phenol, sodium hypochlorite, CPC or CPB achieves, once reconstituted in an aqueous solution, a concentration of at least 0.1%, 0.1%, 0.5%, 0.1% and 0.1 %, respectively.

18. A composition as defined in claim 1, which further comprises a biofilm dislodging enhancer agent.

19. A composition as defined in claim 2, which further comprises a biofilm dislodging enhancer agent.

20. A composition as defined in claim 18, wherein said enhancer agent is a calcium chelator.

21. A composition as defined in claim 19, wherein said enhancer agent is a calcium chelator.

22. A composition as defined in claim 20, wherein said calcium chelator is EDTA in an acid or salt form which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any calcium chelator having a chelating potency substantially equivalent thereto.

23. A composition as defined in claim 21, wherein said calcium chelator is EDTA in an acid or salt form which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.25 % or any calcium chelator having a chelating potency substantially equivalent thereto.

24. A composition as defined claim 18 wherein said enhancer agent is a chaotropic agent.

25. A composition as defined claim 19 wherein said enhancer agent is a chaotropic agent.

26. A composition as defined in claim 24, wherein said chaotropic agent is SDS which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1 % or any chaotropic agent having a chaotropic potency substantially equivalent thereto.

27. A composition as defined in claim 25, wherein said chaotropic agent is SDS which achieves, once reconstituted in an aqueous solution, a

concentration of at least about 0.1 % or any chaotropic agent having a chaotropic potency substantially equivalent thereto.

28. A composition for removing a biofilm from a surface, which comprises an effective dislodging amount of a detergent and an effective dislodging amount of an acid or a salt of an acid; said detergent being selected from sodium dodecyl sulfate, sodium n-decyl diphenylether disulfonate, sodium cocoyl sarcosinate, polyoxyethylene sorbitan monolaureate, cetylpyridinium bromide and cetylpiridinium chloride; said acid being selected from the group consisting of mandelic, 2-ketoglutaric, acetic, iminodiacetic, mucic, glycolic, fumaric, lactic, aspartic, phosphoric, pyruvic, chloroacetic, oxalic, citric, oxamic, malic, dichloroacetic, phenylacetic, benzylic, maleic, succinic, chloromandelic, glutamic, nitrilotriacetic, boric, adipic, formic, glucuronic, salicylic, benzoic, benzoyl formic, phthalic, ketopimelic, ethylenediamine tetraacetic, N-(hydroxyethyl) ethylenediamine triacetic acids, alanine, serine, tryptophane, tyrosine, bicine, tricine and glycine, with the proviso that said composition is neither a mixture achieving a final concentration of SDS 1 % - 2 % and EDTA 1%, of SDS 1% - 2% and mandelic and lactic acids, each at an individual concentration of 1% or in a combined concentration of 2%, of SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%, nor a mixture of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA, all percentages representing final weight per volume concentrations.

29. A composition as defined in claim 28, further comprising a bactericide selected from mandelic acid, phenol, sodium hypochlorite, hydrogen peroxide, CPC and CPB.

30. A composition for removing a biofilm from a surface, which achieves, once reconstituted in an aqueous solution, a concentration of at least about 0.1% but less than 1% SDS, about 0.1% - 1% acid or a salt of an acid and at least about 0.25% but less than 1% EDTA, said acid being selected one or more of 2-ketoglutaric, mandelic, iminodiacetic, mucic, glycolic, fumaric, L-aspartic, phosphoric, pyruvic, chloroacetic acids and DL-alanine.

31. A composition as defined in claim 30, further comprising a bactericidal amount of a bactericide.

32. A composition for removing a biofilm from a surface, which achieves, once reconstituted in an aqueous solution, a concentration of at least about

0.1% SDS, at least about 0.1% acid or a salt of an acid, and at least about 0.25% EDTA, said acid being of 2-ketoglutaric, iminodiacetic, mucic, glycolic, fumaric, aspartic, phosphoric, pyruvic, chloroacetic acids and alanine.

33. A composition as defined in claim 32, further comprising a bactericidal amount of a bactericide.

34. A composition as defined in claim 31, wherein said bactericide is hydrogen peroxide at a final concentration of about 5%, or phenol at concentration of at least about 0.1%, or sodium hypochlorite at concentration of at least about 0.5%, or CPC or CPB at concentration of at least about 0.5%.

35. A composition as defined in claim 33, wherein said bactericide is hydrogen peroxide at a final concentration of about 5%, or phenol at concentration of at least about 0.1%, or sodium hypochlorite at concentration of at least about 0.5%, or CPC or CPB at concentration of at least about 0.5%.

36. A composition which, once reconstituted in an aqueous solution, achieves a final concentration of at least 0.5% CPC or CPB, 1% EDTA, 1% an acid or a salt of an acid selected from mandelic, glycolic, fumaric, citric and phosphoric acids or a mixture thereof, and a buffering agent to achieve a pH of about 7.5 or higher.

37. A method for removing a biofilm from a surface, which comprises the step of contacting said surface with a composition as defined in any one of claims 1 to 36, or with a composition achieving a final concentration of SDS 0.25%, sodium benzoate 2% and sodium salicylate 0.2%, or with a composition achieving a final concentration of 0.1 - 0.3% SDS or SDDD, 0.1 - 0.3% SCS or SLS, 0.1% zinc sulfate, acetate, nitrate or gluconate salts and 0.1 - 0.3% HEEDTA, EDTA or DTPA for a time sufficient to dislodge said biofilm.

38. A method as defined in claim 37, wherein said time is at least about one hour.

39. A method as defined in claim 37, wherein said time is comprised between about 1 and about 18 hours.

40. A method as defined in claim 37, wherein no mechanical aid is required to remove the biofilm.

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Attorney Docket No. 9555.87USWO

MERCHANT & GOULD P.C.

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: IMPROVED BACTERICIDAL AND NON-BACTERICIDAL SOLUTIONS FOR REMOVING BIOFILMS

The specification of which
a. ☐ is attached hereto
b. ☒ was filed on May 3, 2001 as application serial no. 09/831,075 and was amended on (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/CA99/01065 filed November 8, 1999 and as amended on (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.
b. ☒ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	**DATE OF FILING (day, month, year)**	**STATUS (patented, pending, abandoned)**
09/187,249	November 6, 1998	pending

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	**DATE OF FILING** (Day, Month, Year)

I acknowledge the duty to disclose information that is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (reprinted below):

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

(e) In any continuation-in-part application, the duty under this section includes the duty to disclose to the Office all information known to the person to be material to patentability, as defined in paragraph (b) of this section, which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

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